

WE CLAIM:

1. A method for synthesizing an oligosaccharide comprising the steps of:

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(a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture; and

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(b) enzymatically coupling the donor molecule to the acceptor molecule using a mutant form of glycosidase enzyme to form the oligosaccharide, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, and said mutant enzyme being mutated to replace one of said amino acids having a carboxylic acid side chain with a different amino acid of comparable or smaller size, said different amino acid having a non-carboxylic acid side chain characterized in that, said glycosyl donor molecule having a β configuration and said glycoside acceptor molecule having a α configuration, or vice versa.

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2. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry retaining enzyme in which one of the carboxylic acid side chains in the active site functions as an acid/base catalyst and the other carboxylic acid side chain functions as a nucleophile, and wherein the amino acid having the nucleophile carboxylic acid side chain is replaced in the mutant enzyme.

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3. The method of claim 2, wherein the enzyme is a β -glycosidase.

4. The method of claim 3, wherein the glycosyl donor molecule is an α -glycosyl fluoride.

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5. The method of claim 4, wherein the α -glycosyl fluoride is an α -glucosyl fluoride.

6. The method of claim 4, wherein the α -glycosyl fluoride is an α -galactosyl fluoride.

7. The method of claim 1, wherein the enzyme is a β -glycosidase.

8. The method of claim 1, wherein the enzyme is a β -glucosidase.

9. The method of claim 8, wherein the enzyme is *Agrobacterium* β -glucosidase in which amino acid 358 has been changed from glutamic acid to an amino acid with a non-carboxylic acid side chain.

10. The method of claim 8, wherein the enzyme is *Agrobacterium* β -glucosidase in which amino acid 358 has been changed from glutamic acid to alanine.

11. The method of claim 1, wherein the acceptor molecule is an aryl-glycoside.

12. The method of claim 11, wherein the acceptor molecule is a nitrophenyl-glycoside.

13. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry inverting enzyme in which one of the carboxylic acid side chains in the active site functions as an acid catalyst and the other carboxylic acid side chain functions as a base catalyst, and wherein the amino acid having the carboxylic acid side chain which functions as a base catalyst is replaced in the mutant enzyme.

14. The method of claim 1, wherein the enzyme is a mutant form of human or porcine α -amylase in which amino acid 197 has been changed from aspartic acid alanine.

5 15. The method of claim 1, wherein the enzyme is a mutant form of human or porcine α -amylase in which amino acid 197 has been changed from aspartic acid to an amino acid with a non-carboxylic acid side chain.

10 16. The method of claim 1, wherein the enzyme is a mutant form of yeast α -glucosidase in which amino acid 216 has been changed from aspartic acid to alanine.

15 17. The method of claim 1, wherein the enzyme is a mutant form of yeast α -glucosidase in which amino acid 216 has been changed from aspartic acid to a non-carboxylic acid amino acid.

20 18. A mutant form of glycosidase enzyme, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, one of said carboxylic acid side chains functioning as a base catalyst and one of said carboxylic acid side chains
25 functioning as an acid catalyst, and said mutant form of the enzyme being mutated to replace the amino acid residue having the carboxylic acid side chain functioning as a base catalyst with an amino acid having a non-ionizable side chain of comparable or smaller size.

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19. A mutant form of human or porcine α -amylase in which the aspartic acid at position 197 is replaced with a different amino acid having a non-carboxylic acid side chain such that the enzyme cannot catalyze the hydrolysis
35 of oligosaccharides.

20. The mutant amylase of claim 19, wherein the different amino acid is alanine.

21. A mutant form of yeast α -glucosidase in which the
5 aspartic acid at position 216 is replaced with a different amino acid having a non-carboxylic acid side chain such that the enzyme cannot catalyze the hydrolysis of oligosaccharides.

10 22. The mutant α -glucosidase of claim 21, wherein the different amino acid is alanine.

23. An oligosaccharide prepared by the steps of:

15 (a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture; and

(b) enzymatically coupling the donor molecule to the acceptor molecule using a mutant glycosidase enzyme to form
20 the oligosaccharide, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, and said mutant enzyme being mutated to replace one of the amino acid residues having a
25 catalytically active carboxylic acid side chain as a side chain with an amino acid having a non-carboxylic acid side chain, characterized in that, said glycosyl donor molecule having a β configuration and said glycoside acceptor molecule having a α configuration, or vice versa.

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24. The oligosaccharide of claim 23, wherein the glycosidase enzyme is a stereochemistry retaining enzyme in which one of the carboxylic acid side chains in the active site functions as an acid/base catalyst and the other
35 carboxylic acid side chain functions as a nucleophile, and wherein the amino acid having the nucleophilic carboxylic

acid side chain is replaced in the mutant enzyme by an amino acid having a side chain of comparable or smaller size.

5 25. The oligosaccharide of claim 24, wherein the enzyme is a β -glycosidase.

26. The oligosaccharide of claim 25, wherein the glycosyl donor molecule is an α -glycosyl fluoride.

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27. The oligosaccharide of claim 26, wherein the α -glycosyl fluoride is an α -glucosyl fluoride.

15 28. The oligosaccharide of claim 26, wherein the α -glycosyl fluoride is an α -galactosyl fluoride.

29. The oligosaccharide of claim 25, wherein the enzyme is an α -glucosidase.

20 30. The oligosaccharide of claim 24, wherein the enzyme is Agrobacterium β -glucosidase in which amino acid 358 has been changed from glutamic acid to alanine.

25 31. The oligosaccharide of claim 24, wherein the acceptor molecule is an aryl-glycoside.

32. The oligosaccharide of claim 31, wherein the acceptor molecule is a nitrophenyl-glycoside.